Prey stoichiometry and phytoplankton and zooplankton composition influence the production of marine crustacean zooplankton

Running head: Stoichiometry influences marine copepod production.

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Abstract. Manipulative laboratory studies provide strong evidence that phytoplankton primary production (PP), stoichiometry, and taxonomic composition affect marine copepod production (CP), which is the biomass-dominant zooplankton group. However, field observations investigating the simultaneous effects of prey stoichiometric quality, PP, and phytoplankton and copepod taxonomic composition on CP remain relatively rare. Here, we examined how *in situ* CP is affected by carbon:nitrogen:phosphorus (C:N:P) molar ratios of prey, PP, and phytoplankton and copepod composition in the East China Sea (ECS) and Dongsha Atoll in the South China Sea. Field estimates of CP were measured directly as the product of *in situ* instantaneous growth rate estimates by artificial cohort method and copepod biomass. We found that CP was low when prey C:N and C:P ratios were high, but the variation of CP was large when prey C:N and C:P ratios were low. CP did not, however, show a strong positive relationship with PP. Multivariate regression indicates that prey C:N ratio explains most of the variation of CP, followed by phytoplankton and copepod compositions, while PP exerts a weak influence on CP. Our findings suggest that copepod community production is affected by prey stoichiometry, with further modification by copepod and phytoplankton compositions in the ECS. However, the total variance explained by those key factors is less than 50 %, indicating that marine copepod growth and biomass production are influenced by complex interactions in nature.

Keywords: Ecological stoichiometry; subtropical marine copepod production; artificial cohort method; *in situ* incubation; phytoplankton and copepod composition.

1. Introduction

Copepods represent the dominant zooplankton group and serve as an important trophic link between primary producers and higher trophic levels in the ocean (Alcaraz and Calbet, 2007). Identification of which factors influence copepod production (CP; mg C m^{-3} d^{-1}) is essential to understanding the dynamics of pelagic food webs in the oceans. Measuring egg production rate and biomass (assuming a constant growth rate) are two popular approaches used to assess CP in field studies (e.g. Castonguay et al., 2008; Hay et al., 1991; Hopcroft et al., 1998; Kiørboe and Nielsen, 1994; Mayor et al., 2009). However, egg production rate does not fully represent CP and copepod growth rate is not constant under different resource conditions. Using egg production and biomass as the indexes of CP prompts different ecological interpretation. Egg production rates indeed reflect a part of CP, but this measurement does not include somatic growth during naupliar and copepodite stages (Hirst and McKinnon, 2001). Biomass production rates, that is the product of biomass and somatic growth rate, reflect the flux of carbon and energy as the trophodynamic currency from phytoplankton to copepods (Sheldon et al. 1977, Longhurst 1984). The production of copepods may not be supported in the long term if egg production rate or biomass is high but somatic growth rates are slow. Thus, we need studies of *in situ* copepod community somatic growth-based production rate in order to better understand energy transfer in pelagic food webs.

Copepod growth and production rates can be limited by prey carbon supply, i.e., phytoplankton availability (Campbell et al., 2001; Hirst and Bunker, 2003). Thus, primary production (PP; mg C m^{-3} d^{-1}) should be important as a factor supporting copepod production (CP; mg C m^{-3} d^{-1}). However, copepod production is not only influenced by phytoplankton carbon supply, but also by the nitrogen content of phytoplankton (Kiørboe 2007). Thus, apart from phytoplankton carbon supply, stoichiometric constraints (the relative imbalance of elemental composition between consumers and prey) may limit copepod growth and production. Previous studies have indicated that crustacean consumers can grow efficiently only within an optimal stoichiometric range of their prey (e.g. Laspoumaderes et al. 2015) and tend to be more stoichiometrically homeostatic than phytoplankton (Acharya et al., 2004; Sterner and Elser, 2002). The stoichiometric knife-edge hypothesis predicts that crustacean consumers expend energy in order to respire and excrete assimilated elements in excess of their homeostatic demands (i.e. suboptimal prey C:N:P ratio; Boersma and Elser, 2006; Elser et al., 2016; Hessen et al., 2004). Occasionally, when phytoplankton C:N and C:P ratios are too low, crustacean consumers can be carbon-limited and must excrete the excess N and P. Under these conditions, crustacean growth also decreases.

Nitrogen (N) and phosphorus (P) play essential but distinct roles in animal growth and development (Elser et al., 2000). N is key to maintaining proteinaceous body structures, whereas

P is essential to forming the rRNA backbone and thus controls protein synthesis and by extension, organism growth rate (Vrede et al., 2004). Given the physiological roles of both elements, low prey N and P contents lead to low rates of protein synthesis and rRNA production for crustacean zooplankton and consequently hinder their biomass production and growth (Giani, 1991; Vrede et al., 2002). In addition, N- and P-deficient growth are not independent: under N-deficiency, the amounts of P and rRNA are decoupled from growth rate (Acharya et al., 2004). Thus, investigating the link between both N and P supplies versus CP provides a more detailed insight into CP variation. Considering the more strict stoichiometric homeostasis of crustacean consumers, we expect that CP exhibits a unimodal pattern relative to prey C:N and C:P ratios.

In addition to the elemental stoichiometry of prey, the composition of biomolecules such as fatty acids may further constrain crustacean growth and development (Müller-Navarra, 2008). Adding supplementary essential fatty acids or phytoplankton species with highly unsaturated fatty acids can improve crustacean growth (Brett and Müller-Navarra, 1997; Ferrão-Filho et al., 2003). Also, different copepod taxa and their life stages vary in growth strategy and responses to prey stoichiometry (Laspoumaderes et al., 2010; Villar-Argaiz et al., 2002), indicating that copepod taxonomic and stage composition may also influence CP. Thus, we should consider the effects of phytoplankton composition, copepod composition and copepod life stage structure on CP.

Understanding the link between CP versus PP, prey stoichiometry and plankton assemblage in natural systems faces at least two challenges: First, estimation of *in situ* growth-based CP requires intensive effort for onboard incubations and analyses (Runge and Roff, 2000); Second, a broad sampling area and/or time range should be covered to encompass sufficient light and trophic gradients (Finkel et al., 2006). Here, we addressed these challenges by applying the 'improved' artificial cohort method (Lin et al., 2013b; McKinnon and Duggan, 2003) to measure copepod community growth and production rates in the East China Sea and around the Dongsha Atoll in the South China Sea. Both growth and production rate estimates were accompanied by measurements of prey stoichiometry, PP, and phytoplankton and copepod compositions. The large variation of nutrient supply and sea surface light intensity in the sampling areas ensured sufficient variation in PP, prey stoichiometry, and phytoplankton and copepod assemblage compositions for this study (Table A.1). Given the broad set of conditions, we were able to test the following hypotheses: (H1) CP decreases when prey molar C:N and C:P ratios are high (excessive C supply) or too low (excessive N and P supply) according to the stoichiometric knife-edge; (H2) CP increases with PP and phytoplankton biomass according to classical bottom-up effects of prey availability; and (H3) phytoplankton and copepod compositions affect CP.

2. Materials and methods

2.1. Estimation of in situ copepod growth rate and production

We conducted 54 experiments during 2009-2016. The sampling cruises were mainly in the southern East China Sea, while cruises in the northern East Chinas Sea and around the Dongsha Atoll were also included (Fig. A.2). Growth (d^{-1}) and production (mg C m^{-3} d^{-1}) rates were estimated for copepod communities, which account for about 70% of the mesozooplankton biomass in the study area (Tseng et al., 2012). We applied the 'improved' artificial cohort method (see modification of McKinnon and Duggan, 2003 by Lin et al., 2013b) to estimate the specific growth rate GR_i for each juvenile group, where *i* corresponds to each of five copepodite groups (calanoid, cyclopoid (copepods that belong to Cyclopoida but are not corycaeid or oncaeid), corycaeid, oncaeid, and harpacticoid copepodites) and three naupliar groups (calanoid, cyclopoid, and harpacticoid nauplii). We carried out shipboard incubations of two size fractions, 50-80 μm (nauplius) and 100-150 μm (copepodite) copepods, in 3 replicates of 20-L collapsible polyethylene cubitainers. We collected the natural assemblage of phytoplankton smaller than 50 μm and excluded large phytoplankton for juvenile copepod incubations, assuming that nauplii and copepodites of our target size ranges mainly feed on small prey of about 10 μm (considering an optimal predator to prey ESD ratio of 18; Hansen et al. 1994). Seawater with phytoplankton at 10-m depth was collected using 20-L Go-Flo bottles, and screened it through 50-μm mesh prior

to filling of cubitainers to ~90% capacity. Seawater accompanying the size-fractionated copepods made up the remaining volume of the 20-L cubitainers (Fig. A.1 in Appendix A).

We used two Norpac nets (50- and 100-μm mesh) to collect live animals (mainly copepods) for size-fractionated incubations. At each sampling site, the nets were set to 10-m depth and allowed to drift with the ship for 5−10 min. The contents of each net were carefully re-suspended in buckets filled with pre-screened incubation seawater. After gentle mixing, the contents of the 50-μm net were reverse-filtered through an 80-μm mesh and siphoned (~2 L) into cubitainers for the 50−80 μm artificial cohort incubations. Another subsample from the 80-μm mesh reverse filtrate was preserved in 5% formalin-buffered seawater to calculate the biomass distribution at the start of the incubation. The same process was applied to the contents of the 100-μm mesh net, but reverse-filtered through a 150-μm mesh, to establish the 100−150 μm artificial cohort. In each cubitainer, there were typically 500-2000 individuals in total and always more than 50 individuals for each dominant copepod group (50 individuals are the minimum amount for 20 L incubations; Kimmerer et al., 2007; Liu and Hopcroft, 2007). The *in situ* density of copepodites and nauplii in our sampling area is 260 (standard deviation = 511) individuals/20 L and 411 (standard deviation = 705) individuals/ 20 L, respectively. The incubated copepodite and naupliar density is indeed higher than *in situ* density, but previous study showed that the growth of these juveniles is not likely to be inhibited by higher density (Lin et al., 2013b). All cubitainers were

incubated in 200-L dark black tanks filled with circulating seawater pumped constantly from the sea surface layer (Fig. A.1 in Appendix A).

We set the incubation time to 24 h for the 50−80 μm and 48 h for the 100−150 μm size fraction in order to ensure that growth was measurable (Lin et al., 2013a). The environment in the cubitainers was assumed to be similar to *in situ* conditions during the incubation, except that the tanks were always kept dark during incubation. Such a design aims to limit the growth of primary producers during incubation. At the end of shipboard experiments, we terminated the incubations and preserved the individuals in 5% formalin-buffered seawater (Lin et al., 2013a).

We estimated the body size distribution of copepodites and nauplii at the start and end of incubations. For samples collected early in the study period (29 samples), we used a dissecting microscope. We identified and enumerated copepod groups in the preserved samples (in total ~500 individuals), and imaged individuals with a CCD camera mounted to the microscope (Olympus DP71 with the software analySIS LS Starter 2.6). From these copepod digital images, we measured individual body length and width and calculated the biovolume of each individual nauplius and copepodite as: Biovolume (μm^3) = total body length × $width^2$ and Biovolume (μm^3) = prosome length × $width^2$, respectively (Lin et al., 2013a; Wong et al., 2017). For samples collected later (25 samples), we measured body size automatically with the FlowCAM. We set the FlowCAM to the 4X objective (effective magnification = 40X) and 300-μm flow cell

(optimal for size range 30−300 μm) to capture the copepodite and naupliar images in autoimage mode. We then transformed the area-based diameter (ABD) biovolume to equivalent microscope-measured biovolume applying the equations reported by Wong et al. (2017). This transformation ensures that the growth rates estimated by the two methods are comparable in our study. We then calculated specific growth rates (d^{-1}) of all juvenile groups based on the assumption of exponential growth (Lin et al., 2013a) as follows: $\mathrm{GR}_i = \ln\left(\frac{W_{iT}}{W_{i0}}\right)/T$, where W_{i0} and W_{iT} are the modes of biovolume (μm^3) at the start and end of each incubation, respectively, and T is the incubation time: 1 day (24 hours) for the 50−80 μm and 2 days (48 hours) for the 100−150 μm size fraction (Lin et al. 2013b).

Some issues and caveats associated with our *in situ* artificial cohort incubations and efforts to account for and minimize them are worth discussion. We selected specific size ranges (50-80 and 100-150 μm) to represent the nauplius and copepodite cohorts by reverse filtration (McKinnon and Duggan, 2003; Runge and Roff, 2000), but small numbers of adult individuals might still leak into the incubation (<10 adults per 1000 individuals at both the beginning and end of incubation). We have excluded these adult individuals in the same proportion counted at the beginning and end of incubation. All other adults in the end of incubation were retained in our growth calculations. Furthermore, using peak instead of mean body size, we were able to avoid the influence of extreme body sizes in the cohort (Lin et al., 2013b). One potential source of bias

that we were not able to specifically quantify is mortality during incubations. We did observe and exclude the individuals that showed signs of decomposition when analyzing the samples, but most individuals were alive during incubation. In addition to mortality, competition among copepods and other zooplankton may also influence the growth rate, though the low density of non-copepod species and sufficient food and space conditions in the cubitainers should serve to reduce these effects.

To eliminate effects of temperature on growth, we standardized all the GR_i to 20 °C with the Van't Hoff-Arrhenius equation (E = 0.6 eV; based on the review of Brown et al., 2004). To calculate copepod community production rates, we measured the biomass (B_i) of each copepod group. To obtain group-specific biomass, we collected copepodites and nauplii with a 50 μm mesh Norpac net equipped with a mechanical flow meter (HYDRO-BIOS) and preserved the samples in 10 % formalin-buffered seawater. We sorted and counted the number of individuals for each group. Volumes filtered by the nets were estimated from the flow meter and applied to group-specific abundances to calculate density (ind m^{-3}). Densities were then multiplied by mean C biomass for each group. The mean C body mass of each copepod group was calculated from the individual biovolume distribution at the start of each incubation. Copepod biovolume B_i was transformed to wet weight (WW) following Svetlichny (1983): WW = Kc × Biovolume, where Kc is 0.6 for calanoids and 0.705 for cyclopoids (see McKinnon and Duggan 2003), and 0.65 for

groups where conversion factors were not available. The wet weight was then transformed to C body mass (CB) by dry weight (DW) = 0.135 × WW (Postel et al., 2000) and CB = 0.42 × DW (Beers, 1966). Assuming exponential growth, the daily group-specific biomass increment of group-specific production $\mathrm{CP}_i = B_i(e^{\mathrm{GR}i} - 1)$ and community $\mathrm{CP} = \sum_i \mathrm{CP}_i$.

2.2. Prey stoichiometry, biomass and PP measurements

As a proxy for prey (mainly phytoplankton) stoichiometry, we measured the C:N:P molar ratio of particulate organic matter (POM) from the euphotic zone, where photosynthesis and grazing are focused. We collected POM < 50 μm from 50-μm sieve-filtered water samples (5 L water was filtered from 20 L Go-Flo bottles each depth; sampling 4 depths in the euphotic zone) onto pre-combusted GF/F papers (500°C, 6 hours), and froze the POM samples at −20 °C on board. Prior to elemental analysis, samples were acidified and dried for at least 24 hours to remove inorganic carbon. We used an elemental analyzer (EA1108, Fisons, Italy, and FLASH 2000, Thermo SCIENTIFIC, USA) to measure the C and N content in the POM. The P content was measured using molybdate spectrophotometric analysis following wet digestion of POM samples with nitric acid (Parsons et al., 1984). The P content in 17 of the 54 samples was below detection limit. The prey C:N:P molar ratios were then calculated from the C, N, and P contents in POM. Finally, PP (mg C m^{-3} d^{-1}) was estimated with an on-board carbon radioisotope

incubation method (Gong and Liu, 2003).

2.3. Assessing phytoplankton and copepod compositions

In addition to prey stoichiometry and PP, we also considered the taxonomic/group compositions of copepods and phytoplankton as potential factors affecting CP. Phytoplankton composition (biovolume composition of morphological groups) from the euphotic zone was enumerated for only 34 of the 54 experiments. Therefore, only those 34 experiments were included in the following multiple linear regression analysis (section 2.4). We used the biovolume composition of phytoplankton because phytoplankton biomass, instead of abundance, limits zooplankton growth. The relative biovolume of phytoplankton group *i* was calculated as:

$$R_{iB} = \frac{\sum_m \mathrm{Bio}V_{i,m}}{\sum_{j\neq \mathrm{detritus}} \sum_n \mathrm{Bio}V_{j,n}},$$

where, $\mathrm{Bio}V_{i,m}$ is the ESD biovolume (μm^3) of cell *m* classified as group *i* (small phytoplankton, medium phytoplankton, large phytoplankton, diatoms, dinoflagellates, ciliates, and cyanobacteria; see Appendix C), and the denominator is the total phytoplankton biovolume excluding detritus. Copepod composition was calculated for all incubations. Here, we focused on abundance composition of the copepod juvenile groups included in the artificial cohort experiment, given that the interactions between copepod juveniles are at the individual level. The abundance ratio of copepod group *i* (copepodites of calanoid, cyclopoid, corycaeid, oncaeid, harpacticoid, and

nauplii; see Appendix C) was calculated as $R_{i\#} = n_i / \sum_j n_j$, where n_i is the abundance (ind m^{-3}) of group *i* and the denominator is the sum of all copepod group abundances. See Appendix C for detailed descriptions of phytoplankton and copepod grouping procedures.

2.4. Statistical analysis

To test if CP decreases with high and/or excessively low prey C:N and C:P ratios (H1), we first plotted CP against prey C:N or C:P ratio. Visually, we saw no evidence of low CP under low prey C:N or C:P ratio, rather we observed a declining trend (Fig. 1). Thus, we applied linear regressions of CP versus prey elemental ratios. To test if CP increases with PP (H2), we applied linear regression of CP versus PP. The regression relationships were estimated using the *lm* functions of the R package *stats*. We further applied quantile regressions (*rq* function in R package *quantreg*) to investigate the relationships between CP versus prey stoichiometry and PP under different levels of CP.

In addition, we employed multivariate linear regression to investigate the relative effects of prey stoichiometry, PP, phytoplankton and copepod compositions on CP (H3). The relative biovolume of dominant phytoplankton groups ($R_{MediumB}$ and R_{LargeB} as medium and large phytoplankton ratios, $R_{DiatomB}$ as diatom ratio) and dinoflagellates (R_{DinoB}) that occasionally bloom, and relative abundance of the dominant copepodite groups ($R_{Cal\#}$ and $R_{Cyc\#}$ as calanoid

and cyclopoid ratios) and nauplii ($R_{Nau\#}$) were incorporated into the full multivariate regression model (see details in Appendix C). We identified the most parsimonious model on the basis of Akaike's information criterion (AIC) following a stepwise model selection using the *stepAIC* function of the R package *MASS*. Furthermore, the relative importance of these variables on CP in the most parsimonious model was assessed by their R^2 contribution using the function *calc.relimp* (calculating the R^2 contribution averaged over orderings among regressors with the lmg method) in the *relaimpo* package.

3. Results

3.1. Does CP decrease with suboptimal prey C:N and C:P ratios (H1)?

Linear regression analyses indicated a statistically significant decline of CP with prey C:N ratio (Fig. 1a; CP = −0.088 Prey C:N+0.768, R^2 = 0.190, p = 0.001, n = 54), but the linear regression of CP versus prey C:P ratio was not significant (Fig. 1b; CP = -9.853×10^{-5} Prey C:P + 0.290, R^2 = 0.042, p = 0.224, n = 37). CP was lower when prey molar C:N and C:P ratios were high (Fig. 1a and C:P ratio > 500 in Fig. 1b), inferring that low N and P in food may limit CP. The biomass of copepods also decreased significantly with increasing prey C:N ratio, showing a similar trend to CP with prey C:N ratio (Fig. 2; Copepod biomass = −0.511 Prey C:N + 4.723, R^2 = 0.085, p = 0.032, n = 54). The quantile regressions indicated that only quantiles ≥ 50 % yielded

significantly negative relationships between CP and prey C:N (50% and 90% quantiles) and between CP and prey C:P (75% quantile) (Fig. B.1a and b).

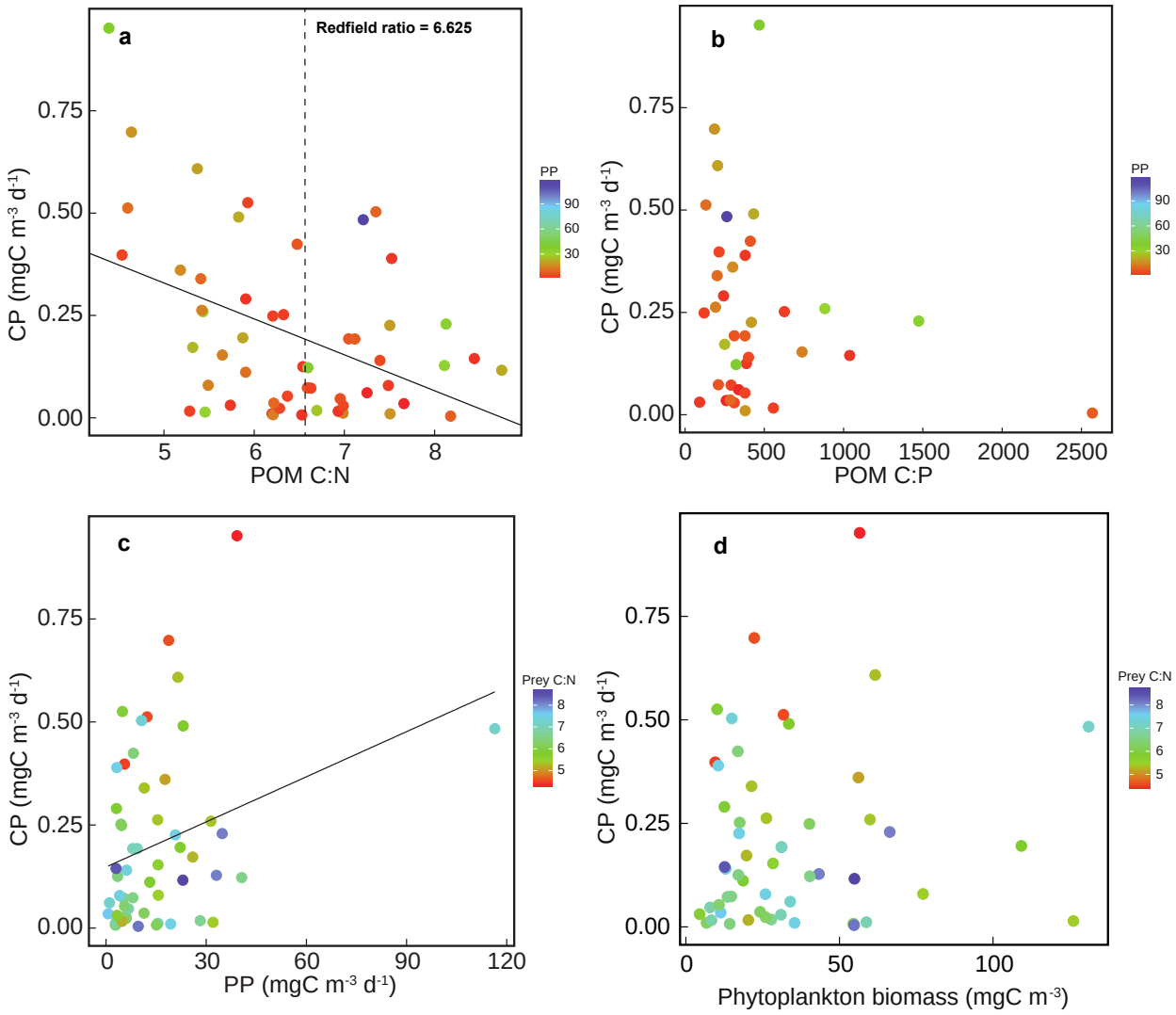

Figure 1. Relationship between copepod production (CP) versus (a) POM C:N ratio, (b) POM C:P ratio, (c) PP, and (d) phytoplankton biomass. The vertical dashed line in (a) indicates the Redfield molar C:N ratio. We depicted PP (mg C m^{-3} d^{-1}) by color on the CP versus C:N (a) and C:P ratio (b) scatter plot, and C:N ratio (mole:mole) on the CP versus PP and phytoplankton biomass scatter plot (c and d) to visualize the additive effects of prey stoichiometry and PP on CP. The solid lines ($p < 0.05$) represent the results of linear regression.

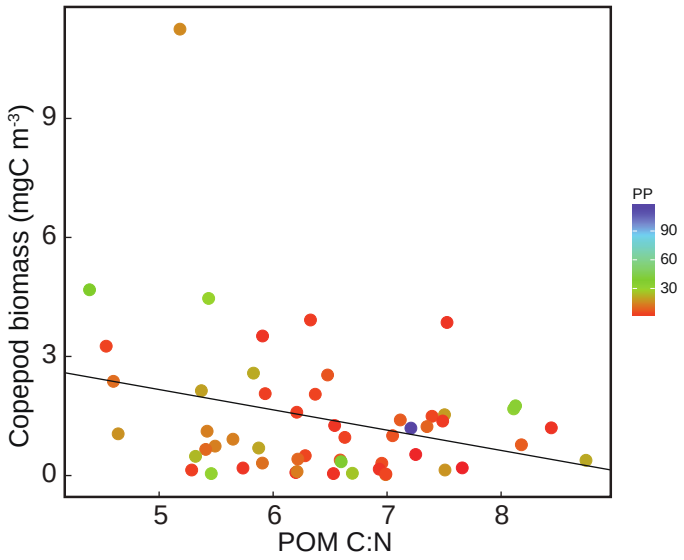

Figure 2. Relationship between copepod community carbon biomass versus POM C:N ratio. The color of the symbols represents PP (mg C m^{-3} d^{-1}). The solid line represents the result of linear regression ($p < 0.05$).

3.2. Does CP increase with high PP (H2)?

CP was weakly correlated with PP (Fig 1c; CP = 0.004 PP + 0.148, R^2 = 0.095, p = 0.024, n = 54). However, the regression was non-significant after removing an extremely high PP (> 100 mg C $m^{-3}d^{-1}$) outlier value (CP = 0.005 PP + 0.129, R^2 = 0.072, p = 0.052, n = 53). Moreover, the quantile regressions were not significant for any quantile (Fig. B.1c), and CP was neither significantly correlated with phytoplankton biomass (Fig. 1d).

3.3. Is CP influenced by copepod and phytoplankton compositions (H3)?

Along with prey C:N ratio (the main stoichiometric ratio that influences CP) and PP, we incorporated the relative biovolume of dominant phytoplankton groups, and the relative abundance of the dominant nauplii and copepodite groups into the multivariate model (Appendix C). The most parsimonious model explaining variation of CP included: prey molar C:N ratio (Prey C:N), cyclopoid copepodite abundance ratio ($R_{Cyc\#}$), and dinoflagellate biovolume ratio (R_{DinoB}) (Table 1; the best multivariate linear model: CP = −0.182 Prey C:N – 1.092 $R_{Cyc\#}$ + 0.602 R_{DinoB} + 1.385, R^2 = 0.418, p = 0.001, n = 34). The relative importance of the three variables followed in order as: Prey C:N > $R_{Cyc\#}$ > R_{DinoB} (Table 2).

To explore what may explain the high variation of CP when the prey molar C:N ratio is below Redfield (< 6.625), we applied stepwise multiple linear regression to explain CP considering the relative abundance of copepod groups. We found that the model includes only the naupliar abundance ratio (considering only data of prey C:N ratio < 6.625: CP = 0.579 $R_{Nau\#}$ − 0.110, R^2 = 0.130, p = 0.043, n = 32; Table 3). This suggests that the dominance of nauplii increases CP when prey N is replete.

Initial model: CP = Prey C:N + PP + $R_{MediumB}$ + R_{LargeB} + $R_{DiatomB}$ + R_{DinoB} + $R_{Cal\#}$ + $R_{Cyc\#}$ + $R_{Nau\#}$	
Model	AIC
Initial full model	−95.37
Prey C:N + $R_{MediumB}$ + R_{LargeB} + $R_{DiatomB}$ + R_{DinoB} + $R_{Cal\#}$ + $R_{Cyc\#}$ + $R_{Nau\#}$	−97.35
Prey C:N + $R_{MediumB}$ + R_{LargeB} + + R_{DinoB} + $R_{Cal\#}$ + $R_{Cyc\#}$ + $R_{Nau\#}$	−99.20
Prey C:N + R_{LargeB} + R_{DinoB} + $R_{Cal\#}$ + $R_{Cyc\#}$ + $R_{Nau\#}$	−100.94
Prey C:N + R_{DinoB} + $R_{Cal\#}$ + $R_{Cyc\#}$ + $R_{Nau\#}$	−102.66
Prey C:N + R_{DinoB} + $R_{Cal\#}$ + $R_{Cyc\#}$	−102.83
Prey C:N + R_{DinoB} + $R_{Cyc\#}$	−103.45
Most parsimonious model: CP = 1.385 − 0.182 Prey C:N + 0.602 R_{DinoB} − 1.092 $R_{Cyc\#}$ AIC = −103.45	

Table 1. AICs of multivariate linear regressions and stepwise model selection for investigating the factors that determine copepod production (CP). $R_{MediumB}$, R_{LargeB}, $R_{DiatomB}$ and R_{DinoB} represents the biovolume ratios of medium, large phytoplankton, diatoms, and dinoflagellates to total phytoplankton biomass. $R_{Cal\#}$, $R_{Cyc\#}$, and $R_{Nau\#}$ represents the abundance ratio of calanoid copepodites, cyclopoid copepodites, and all nauplii to total copepod abundance. There are 34 sets of experiments with complete CP, stoichiometry, PP, and plankton composition data.

Variable	Relative importance
Prey C:N	0.762
$R_{Cyc\#}$	0.191
R_{DinoB}	0.047

Table 2. Relative contributions of variables explaining the variation of copepod production (CP) in the most parsimonious model. The relative importance is the R^2 contribution to each regressor based on lmg (Lindeman et al., 1980).

Initial model: $CP = R_{Cal\#} + R_{Cyc\#} + R_{Nau\#}$	
Step	AIC
	−68.52
$R_{Cyc\#} + R_{Nau\#}$	−69.22
$R_{Nau\#}$	−70.56
Most parsimonious model: $CP = 0.579\ R_{Nau\#} - 0.110$	
AIC= -70.56	

Table 3. AICs of multivariate linear regressions and stepwise model selection for investigating the factors that determine copepod production (CP) when POM C:N < 6.625 (Redfield ratio). $R_{Cal\#}$, $R_{Cyc\#}$, $R_{Nau\#}$ represents the abundance ratio of calanoid copepodites, cyclopoid copepodites, and all nauplii to total copepod abundance. There are 32 sets of experiments with POM molar C:N < 6.625, CP, and plankton composition data.

4. Discussion

4.1. Prey stoichiometry, especially prey C:N ratio, affects copepod production

We found that copepod production (CP) is lower when prey C:N and C:P is high, indicating that N and P nutrients limit copepod production. It is worth noting that we found a clearer reduction of CP with high prey C:N than with high C:P ratio, which infers that N limitation seems to be more important to copepod community production rate in our studied marine areas. This may be due to our focus on marine copepods, which are more sensitive to N limitation than cladocerans (Hassett et al., 1997; Sterner and Elser, 2002). Furthermore, CP and copepod biomass were dominated by copepodites (Fig. B.3 and B.4), which have higher N demand than nauplii (that is, copepodites have significantly lower C:N and slightly higher N:P ratio than nauplii, Fig. B.2a and c in Appendix B; also see Villar-Argaiz et al. 2002 and Meunier et al. 2015). Though naupliar abundance is high in tropical copepod communities, naupliar biomass and production rates are usually much lower compared with copepodites, due to the low body mass of nauplii (Hopcroft et al., 1998). Thus, prey N-deficiency that decreases copepodite growth should affect copepod community production more strongly than factors that influence naupliar growth.

We found that low CP is associated with high prey C:N ratio, suggesting that copepod carbon biomass production can be limited by N in our study area. However, we found large variation of

CP when the C:N ratio of prey is below Redfield ratio (C:N = 6.625 in Fig. 1a; Gismervik 1997). This result does not fully correspond to the strict stoichiometric threshold of zooplankton that low prey carbon to nutrient ratio (N-rich prey) can have negative effects on the growth of consumers (Boersma and Elser, 2006; Elser et al., 2016). To find a possible explanation for this inconsistency, we further examined the effect of copepod life stages on CP and found that nauplii abundance exerts a strong effect on CP when prey C:N is lower than Redfield ratio. That is, when naupliar and copepodite growth is not nitrogen-limited, the higher abundance of fast-growing early life stages (i.e. nauplii) would increase the total biomass production of the copepod community (Hopcroft et al., 1998; Hopcroft and Roff, 1998; Hygum et al., 2000; Leandro et al., 2006).

4.2. Primary production influences CP, but is not the main limiting factor for CP

A positive relationship between CP and primary production was observed in the East China Sea and the Dongsha Atoll, suggesting a bottom-up effect of prey availability (Fig. 1c). However, the association between CP and phytoplankton C production (PP) is not as strong as between CP and prey C:N ratio (Fig. 1b and c; Table 1). In particular, at low PP (PP < 30 mg C m^{-3} d^{-1}), we found that high CP occurred with lower prey C:N ratios (Fig. 1c). This indicates that low but N-replete phytoplankton production may support appreciable CP. Whereas previous studies have

reported an interactive influence of phytoplankton P content and PP on the production of freshwater herbivores (Persson et al., 2007; Urabe et al., 2002), we found that sufficient prey N is related to high CP in this subtropical marine system.

4.3. Prey and copepod compositions affect CP

While prey C:N ratio has the highest relative importance in the multivariate model, the abundance ratio of cyclopoid copepodites and the biomass ratio of dinoflagellates also makes significant contributions to variation of CP (Table 1 and Table 2). The negative relationship between CP and cyclopoid copepodites may be due to the lower growth rates of cyclopoid relative to calanoid copepodites (Hirst and Lampitt, 1998; Lin et al., 2013a). The positive relationship between CP and the dinoflagellate biomass ratio is possibly due to the rich fatty acid contents of dinoflagellates (Viso and Marty, 1993), which can support copepod growth. Indeed, the positive influence of dinoflagellates on copepod growth has been supported by diet manipulation experiments, where copepods consuming a mixture of dinoflagellates and microzooplankton grow better than those feeding on a diatom-dominant diet (Nejstgaard et al., 2001). We infer from this result that prey and copepod compositions potentially influence the trophic interactions and CP, as proposed in our third hypothesis. In this study, we have focused on investigating total CP, whereas contrasting the difference in production among copepod

taxonomic groups and stages could be interesting. These taxonomy- and stage-specific analyses will be reported on a separate communication.

4.4. Other trophic interactions and environmental conditions that may determine CP

Though we found that CP decreases with high prey C:N ratios and that it is additionally affected by both prey and copepod compositions, these three factors together explain less than half the variation of CP (R^2 = 0.418). The quantile regressions also showed that only the regressions of quantiles ≥ 50 % are significant (Fig. B.1a; regressions are significant at quantiles 90% and 50%), indicating that stoichiometry alone was not a good explanation for those observations of low CP. Another source of CP variation may be due to the reaction time needed for prey stoichiometry to change. For instance, Malzahn and Boersma (2012) found that exposure to P-limiting food caused long-lasting reduction of copepod growth even after re-feeding with a P-sufficient prey. However, the C:N and C:P ratio of POM < 50 μm we measured is a snapshot of prey stoichiometry at the start of our incubations and does not necessarily reflect what the copepods experienced before incubation. Thus, if copepod growth has not adapted to changes of *in situ* prey stoichiometry, we may see a mismatch between observed prey stoichiometry and CP.

We also note some caveats associated with our measurements of prey C:N and C:P ratio: the

POM < 50 μm that we sampled included phytoplankton as well as microzooplankton and mixotrophic protists (Flynn et al., 2013), and detritus (non-living particles; Postel et al. 2000). Microzooplankton and mixotrophic protists are key organisms in microbial food webs (Azam et al., 1983) and influence energy transfer to mesozooplankton (Calbet and Saiz, 2005), but we did not investigate the effect of the microbial food web in this study. Furthermore, the existence of C-rich detritus (dead biomass) in the POM may increase the C:N or C:P ratio that we observed. Nevertheless, copepods can select living cells which have lower C:N and C:P ratios instead of ingesting dead biomass (Demott, 1988; Paffenhöfer and Sant, 1985), suggesting that the realized prey C:N and C:P ratios consumed by copepods may be lower than the POM C:N and C:P ratio we measured. With our current data, we could not separate the stoichiometric contribution of phytoplankton, microzooplankton, and detritus to POM, and thus in this study we are not able to investigate these hidden trophic interactions.

5. Conclusions

In summary, our study demonstrates prey stoichiometry is a more important predictor of copepod biomass production than primary production in this subtropical marine ecosystem. In particular, when prey molar C:N or C:P ratio is high, CP is low. However, when prey molar C:N or C:P ratio is low, CP variation is large, highlighting the complexity of understanding CP in

natural systems. Furthermore, copepod and phytoplankton compositions influence copepod community production as well. Interestingly, PP explained only a minor portion of the variation in copepod community production in the East China Sea and South China Sea. In conclusion, our *in situ* incubation experiments highlight the knowledge gained by measuring copepod growth and production rates for understanding stoichiometric effects on copepod communities, and also underscores the complexity of variation of copepod production in natural systems.

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Statement for data archiving

We agree to make the data necessary to reproducing our results available and R codes for analysis on Mendeley Data http://dx.doi.org/10.17632/sh683s8mwf.1.

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